screening the hybridomas for binding to the desired apolipoprotein or lipoprotein.

50. (amended) The method of claim [43] 49 for making antibodies to an apolipoprotein wherein the apolipoprotein is selected from the group consisting of Apo AI, Apo AII, Apo B, Apo CIII, and Apo E.

51. (amended) The method of claim [43] 49 for making antibodies to a lipoprotein wherein the lipoprotein is selected from the group consisting HDL, LDL, and VLDL.

Remarks

Rejections under 35 U.S.C. 112

Claims 48-51 have been rejected on the basis that the claim is enabled only for an antibody made with an antigen purified by electrophoresis. This rejection is respectfully traversed.

Applicants are not claiming the method to purify the antigen. They care claiming a method to produce an antibody. It is applicants' opinion that any number of methods that are well known to those skilled in the art, and which are described in the application for example at pages 42-43 under the heading "VI. Purification of Apolipoprotein", could be used to achieve this purification. However, it is presumably agreed that what is really in issue is the degree of purification of the antigen, not the method by which it is achieved. Accordingly, the claim has been amended to recite that the antigen is free of self-aggregated and degraded material from the delipidated, reduced, carboxymethylated, and solubilized apolipoprotein or lipoprotein, which is presumably the result obtained using polyacrylamide gel electrophoresis. See example 2 at page

47.

The claims have also been rejected as not enabled for polyclonal as well as monoclonal antibodies. No basis for the rejection is provided. It is well established that when an animal is immunized, the result is polyclonal antibodies. This is an inherent, invariable result of the immunization process. This is the step which is defined by claim 48. It is proven by the results in the examples that at least one of the cells which produce antibodies present in the polyclonal antibody mixture in the immunized animal is an antibody as defined by the amended claims, since when the cells were fused to produce hybridomas which could be maintained in culture, one could obtain monoclonal antibodies. The simple fact is that each antibody is reactive solely with a single epitope; so each cell must produce "monoclonal" antibodies. Immunization of an animal to produce only the same type of antibodies, reactive with only a single epitope, has never been achieved. Therefore the claim is enabled for production of polyclonal antibodies which include antibodies having the defined reactivity, as well as monoclonal antibodies. The claims are not missing method steps: the step of immunization produces antibodies.

Claim 48 has been amended to clarify the claimed scope in view of the examiner's concerns. It is clarified that the starting material, the apolipoprotein or lipoprotein, is purified. It is solubilized using a reducing or denaturing agent (such as urea; see example at page 47). Lipoproteins are not soluble in an aqueous solution; they are suspended.

Rejections under 35 U.S.C. 102(b)

Claims 48, 50 and 51 were rejected as disclosed by Lee, et al., Biochimica Biophysica

Acta 666:133-1346 (1981). This rejection is respectfully traversed.

As the examiner correctly notes, Lee teaches isolating lipoprotein, removing lipid with ethanol and diethyl ether, solubilizing the lipoprotein in 6 M guanidine HCl buffer containing a reducing agent, and purification of the apolipoprotein.

Lee, however, does not immunize an animal with the delipidated, decarboxymethylated, reduced apolipoprotein. He has removed the reducing agents from the apolipoprotein.

In contrast, as now more clearly defined by the amended claim, applicants immunized with the delipidated, decarboxymethylated, reduced apolipoprotein from which the degraded and complexed materials had been removed. As is apparent from the example at page 47, lines 20-20, this is what applicants do so that the antibodies are made to antigen presented solely in its reduced, delipidated, decarboxylmethylated form.

Rejection under 35 U.S.C. 103

Claims 48, 49, 50 and 51 were rejected under 35 U.S.C. 103(a) as obvious over Lee, et al., in combination with Gooding, Monoclonal Antibodies, Academic Press, Orlando, FL 1983, p. 56-97. Claims 48, 50 and 51 were rejected under 103 as obvoius over Lee, et al., in combination with Zhou, et al., Acta Acad. Med. Hubei 11(4):298-302 (1990) in combination with Mills,et al., in Laboratory Techniques in biochemistry and molecular biology, Elsevier 1984 p. 384-448. These rejections are respectfully traversed.

Lee is discussed above. Gooding adds nothing more except the methodology to make monoclonal antibodies, which applicants recognize is well known. The combination does not

yield the claimed method, since the combination does not solve the basic problem: how to make an antigen that will yield antibodies immunoreactive with an epitope of an apolipoprotein or lipoprotein which reacts in the same way and to the same degree regardless of the conformation of the protein or the concentration of lipid present.

Zhou also does not solve this problem. As the examiner has stated, Zhou eluted the gels with SDS-Tris buffer, so the Apo AI is not reduced, delipidated, carboxymethylated and solubilized at the time the animal is injected.

Mills does not teach immunization with the reduced, delipidated, carboxymethylated, and solubilized apolipoprotein.

In summary, none of the prior art recognized that it was possible to produce an antibody that reacted with an epitope of an apolipoprotein or lipoprotein, regardless of the conformation of the antigen or the concentration of lipid present, much less provided the means by which one could achieve such antibodies.

Allowance of all claims 48-51, as amended, is earnestly solicited.

Respectfully submitted,

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CERTIFICATE OF UNDER 37 CFR § 1.8(a)

I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being sent by first class mail with adequate postage to the Assistant Commissioner for Patents, Washington, D.C. 20231 on the date shown below.

Jean Hicks

Date: September 4, 2001

APPENDIX: Specification as Amended
Page 1:

METHOD FOR MAKING ANTIBODIES IMMUNOREACTIVE
WITH AN EPITOPE OF AN APOLIPOPROTEIN

This application is a continuation-in-part of U.S.S.N. 08/268,809 filed June 30, 1994, by Eugen Koren and Mirna Koscec, now U.S. Patent No. 6,107,045, and claims priority to PCT/US95/08331 filed June 30, 1995 by Oklahoma Medical Research Foundation.--

Abstract:

METHOD FOR MAKING ANTIBODIES IMMUNOREACTIVE WITH AN EPITOPE OF AN APOLIPOPROTEIN

Abstract of the Disclosure

A method for making an antibody to an epitope of an apolipoprotein or lipoprotein which reacts with the apolipoprotein or lipoprotein independently of lipid content and conformation of the apolipoprotein or lipoprotein has been developed. The antibody is produced by immunizing an animal with the apolipoprotein or lipoprotein which has been delipidated, reduced, carboxymethylated, solubilized, and purified to remove self-aggregated and degraded material from the delipidated, reduced, carboxymethylated, and solubilized apolipoprotein or lipoprotein. The antibodies are useful in diagnostic assays for lipoprotein or apolipoprotein.

APPENDIX: Marked up Copy of the Claims as Amended

48. (three times amended) A method for making [an antibody] antibodies to an epitope of an apolipoprotein or lipoprotein which reacts with the apolipoprotein or lipoprotein independently of lipid content and conformation of the apolipoprotein or lipoprotein, comprising

immunizing an animal with [the] apolipoprotein or lipoprotein which [has been] is delipidated, reduced, carboxymethylated, and solubilized with a reducing or denaturing agent, [and purified to remove] wherein all self-aggregated and degraded material has been removed from the delipidated, reduced, carboxymethylated, and solubilized apolipoprotein or lipoprotein.

49. (amended) The method of claim [43] 48 further comprising

isolating the spleen from the immunized animals,

producing hybridomas from the spleen, and

screening the hybridomas for binding to the desired apolipoprotein or lipoprotein.

50. (amended) The method of claim [43] 49 for making antibodies to an apolipoprotein wherein the apolipoprotein is selected from the group consisting of Apo AI, Apo AII, Apo B, Apo CIII, and Apo E.

51. (amended) The method of claim [43] 49 for making antibodies to a lipoprotein wherein the lipoprotein is selected from the group consisting HDL, LDL, and VLDL.

APPENDIX: Clean Copy of the Claims as Amended

48. (three times amended) A method for making antibodies to an epitope of an apolipoprotein or lipoprotein which reacts with the apolipoprotein or lipoprotein independently of lipid content and conformation of the apolipoprotein or lipoprotein, comprising

immunizing an animal with apolipoprotein or lipoprotein which is delipidated, reduced, carboxymethylated, and solubilized with a reducing or denaturing agent, wherein all self-aggregated and degraded material has been removed from the delipidated, reduced, carboxymethylated, and solubilized apolipoprotein or lipoprotein.

49. (amended) The method of claim 48 further comprising

isolating the spleen from the immunized animals,

producing hybridomas from the spleen, and

screening the hybridomas for binding to the desired apolipoprotein or lipoprotein.

50. (amended) The method of claim 49 for making antibodies to an apolipoprotein wherein the apolipoprotein is selected from the group consisting of Apo AI, Apo AII, Apo B, Apo CIII, and Apo E.

51. (amended) The method of claim 49 for making antibodies to a lipoprotein wherein the lipoprotein is selected from the group consisting HDL, LDL, and VLDL.